Atty. Dkt. No. 048331-1707

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Tabor et al.
Title:	ISOTHERMAL AMPLIFICATION OF DNA
Appl. No.:	10/813,693
Filing Date:	10/7/2003
Examiner:	Bertagna, A.M.
Art Unit:	1637
Conf. No.:	4141

SECOND DECLARATION UNDER 37 CFR § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Stanley Tabor, being duly warned, hereby declare and say that:

1. I have extensive experience as a researcher and scientist in DNA replication, especially with respect to amplification technologies and T7 DNA replication system. I hold the position of Lecturer on Biological Chemistry and Molecular Pharmacology at Harvard University, the assignee of the US Patent Application No. 10/813,693 (the "'693 Application"). I am an inventor or co-inventor on more than 15 issued U.S. patents and more than 100 issued and pending patents worldwide. In addition, I have authored more than 50 scientific publications. I received a B.S. in Biology from Stanford University and a Ph.D. in Biochemistry from the Harvard University. A copy of my Curriculum vitae is attached hereto as Appendix 1.

2. I am an inventor of the above identified patent application.

3. I have read and am familiar with the Office Action dated May 28, 2009 and the claim amendments and response to the Office Action dated May 28, 2009. I have reviewed the cited references of Scherzinger (European Journal of Biochemistry (1977) 72: 543-558), Sorge (US 5,556,772). I understand that in the May 28, 2009 Office Action, the Examiner has rejected the claims as allegedly being unpatentable in view of several references that include Scherzinger and Sorge

4. The instant claims of the '693 application are based at least in part on our discovery that it is possible to amplify DNA to produce an amount of amplified product that is at least 10-fold (or at least 100-, 1,000-, 1,000,000- or 10,000,000-fold amplification) over the input template DNA **without adding exogenous primers** by incubating the template DNA molecule in a reaction mixture including a DNA polymerase and at least two accessory proteins at a constant temperature. As explained in my previous declaration (see, for example paragraph 5 of my declaration executed on February 27, 2009), without knowledge of the experimental results first disclosed in this patent application, researchers in the field would not have predicted or expected that DNA could be amplified at least 10-, 100-, 1,000-, 1,000,000- or 10,000,000-fold in an isothermal reaction without exogenous primers as is required by the claims. In this regard, prior to our discoveries reported in the patent application, it would have been expected that isothermal, primer-free reactions having components such as described in the claims could result at best in DNA replication producing modest amounts of DNA (i.e., less than about five fold) and certainly would not have been expected to result in exponential amplification or in a 10-, 100-, 1,000-, 1,000,000- or 10,000,000-fold amplification.

5. Once I observed the results of the experiments described in the Application (for example, the results of Examples 1 and 2) and the surprising amount of DNA amplified in those experiments, I immediately recognized (as would most researchers in the field of DNA polymerases and DNA replication and amplification) that the level of amplification we observed could be achieved using a wide range of constant temperature exogenous primer-free reaction systems in accordance with the claims—for example in reaction systems involving various types and amounts of DNA polymerases, various types and amounts of accessory proteins as well as

other variations. In this regard, a researcher of average ability in the field of DNA polymerases using the teachings of the application as a guide could readily adjust types and amounts of various components in the reaction systems and achieve the 10-, 100-, 1,000-, 1,000,000- or 10,000,000-fold amplification in accordance with the claims; and upon reviewing the data and teachings of the application would immediately recognize that achieving the claimed level of amplification is possible.

6. Scherzinger focuses on fundamental mechanisms underlying DNA replication, and includes nothing to suggest any method designed for DNA amplification. The method disclosed in Scherzinger results in at most a 4-fold increase in the template DNA (Scherzinger at p.549, col. 2), and because the purpose of Scherzinger is merely an academic investigation of DNA replication, there would be no reason based on Scherzinger alone for one to try to change the method to increase DNA production. Even if one did try to modify the Scherzinger methods, as I explained in paragraph 7 of my February 27, 2009 declaration there would be no expectation that any modifications of Scherzinger could result in a 10-fold (much less a 100-, 1,000-, 1,000,000- or 10,000,000-fold) amplification without exogenous primers as is required by the claims.

7. Sorge discloses methods of DNA amplification that, unlike Scherzinger and the claimed methods, involve PCR (i.e., using temperature cycling) and require exogenous primers (see, for example, the "Summary of the Invention" at column 2, lines 39-41). There were many amplification reactions known in the art, such as the Sorge method, that involved exogenously added primers and were able to obtain a more than 10-fold amplification. However, prior to the our instant application, there was nothing to indicate that such amounts of amplification could be achieved without adding exogenous primers. Because, the Sorge methods require exogenous primers, there is nothing in the reference that would provide any information about the amounts of amplification that could be achieved without exogenously added primers. Moreover, the traditional amplification methods of Sorge (i.e., using exogenous primers in PCR reactions) are entirely different than the physiological replication experiments disclosed in Scherzinger,

therefore, an average researcher would have no reason to combine the methods of the two references.

8. One aspect of our discoveries that is reflected, for example, in instant claims 11, 24, and 132, is our finding that the inclusion of a DNA polymerase having reduced 3' to 5' exonuclease activity (for example in combination with a DNA polymerase having normal 3'-5' exonuclease activity) in a primer free isothermal reaction mixture results in drastically higher levels of amplification (i.e., much higher levels of amplification than replication reactions that occur with only a DNA polymerase with a normal level of exonuclease activity). Without observing the experimental results of application, it could not have been accurately predicted or expected (by us or by any other researchers in the field of DNA polymerases) that the use of a DNA polymerase having reduced 3' to 5' exonuclease activity would cause any substantial increase in DNA synthesis in exogenous primer-free constant temperature reaction systems, much less the dramatic increase we discovered. In this regard, there is nothing in Scherzinger or in any other reference I know of that was published prior to our discovery that discloses, teaches or suggests using a DNA polymerase with a normal level of exonuclease activity and a DNA polymerase modified to have reduced 3' to 5' exonuclease activity in a constant temperature reaction system that does not include exogenously added primers (for example, as in instant claims 11, 24, and 132), nor is there anything that would lead to any reasonable expectation that the use of two such forms of polymerase in a constant temperature reaction system such as Scherzinger that do not include exogenously added primers could result in the dramatically high level of amplification (i.e, at least 10-, 100-, 1,000-, 1,000,000- or 10,000,000-fold amplification) without exogenously added primers as required by the claims.

9. Sorge's amplification reaction includes exogenous primers and a combination of a DNA polymerase with normal exonuclease activity and a DNA polymerase with reduced exonuclease activity, and in explaining the reasons for using the combination states:

> "the composition is especially useful in DNA synthesis when there exists one or more mismatched nucleotide(s), particularly mismatches at the 3' end of one or more synthesis primer(s). In such situations, the results achieved, i.e., the amount of synthesis product produced, are significantly greater than the amount of synthesis product obtained using either a DNA polymerase with less 3'-5'

> exonuclease activity than the enzyme possessing substantial 3'-5' exonuclease activity or with a DNA polymerase possessing substantial 3'-5' exonuclease activity alone."

Sorge at column 3, lines 10-22. Sorge's motivation to include the combination of a DNA polymerase with normal exonuclease activity and another DNA polymerase with reduced exonuclease can be further understood from the examples 2, 3, 4, and 5, each of which include 3'-mismatched primers and a combination of DNA polymerases. In all of these examples, the boost in amplification observed by using the combination of polymerases is attributed to the 3'-5' exonuclease activity of the polymerase and its action with regard to the primer mismatches. It is readily understood that that any advantages disclosed in Sorge for using the DNA polymerase combination is based on the interaction of the polymerases with the exogenously added primers.

10. Because the Scherzinger replication reaction does not include exogenous primers, there would be no reason based on the disclosures of Sorge to modify Sherzinger such as to use the combination a DNA polymerase with exonuclease activity and a DNA polymerase with reduced exonuclease activity. Moreover, without exognously added primers, there would no basis for any expectation that using the DNA polymerase combination in the Scherzinger reaction would result in any increase DNA synthesis, much less an increase sufficient to arrive at the levels required by the claims.

11. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

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Respectfully submitted,

Date November 20, 2009

Stanley Tabor